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Synthesis and Biological Activity of Allosamidin and Allosamidin Analogues*

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Abstract: The chitinase inhibitor/insect ecdysis inhibitor allosamidin and eight allosamidin analogues have been synthesised from simple carbohydrate starting materials. Allosamidin was assayed against *Tineola bisselliella* (Hummel) larvae and all nine compounds were examined for their effects on the development of larvae of *Lucilia cuprina* (Wiedemann). High larval mortality compared to controls resulted when *T. bisselliella* and *L. cuprina* larva were exposed to allosamidin. The $(1 \rightarrow 3)$ linked regioisomer, the dimeric analogue and the *gluco*-configurated dimeric analogue of allosamidin all showed high activity against *L. cuprina* larvae. The regioisomer, the $(1 \rightarrow 3)$ linked isomer and its dimeric analogue, as well as the monomer allosamizoline and its regioisomer, were inactive. These new in-vivo results are consistent with known in-vitro insect chitinase inhibition data, in that greatest larval mortality was exhibited by the best inhibitors.

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1 INTRODUCTION

The allosamidins are a family of mono- and disaccharide glycosides with chitinase inhibitory activity. The parent compound, allosamidin (Fig. 1, **1**) was isolated ten years ago from *Streptomyces* fermentations.[1,2] It contains two *N*-acetylhexosamine residues with the unusual D-*allo*-configuration and a novel aminocyclitol, allosamizoline (**8**), joined by two $(1 \rightarrow 4)$ glycosidic bonds. (For convenience, carbohydrate numbering is used for the cyclitol moiety.) Its unique chemical structure made allosamidin an attractive synthetic target, resulting in the publication, so far, of four total syntheses[3–6] and of five separate preparations of the aglycon **8**.[7–11] Close analogues of allosamidin have been obtained from related microbial sources,[12–14] as well as synthetically[4,6,11,15–17] and semisynthetically.[18]

The biological activity of these compounds likewise met with keen interest, since they were the first and until recently the only known potent chitinase inhibitors[1,2] (a second family of chitinase inhibitors, the styloguanidins,[19] has now been reported). They were tested for inhibition of chitinases from a great variety of sources and for toxicity to the organisms dependent on these enzymes.[1,2,14,20,21] Of particular relevance is their effect on insect chitinases and, *in vivo*, on insect larvae

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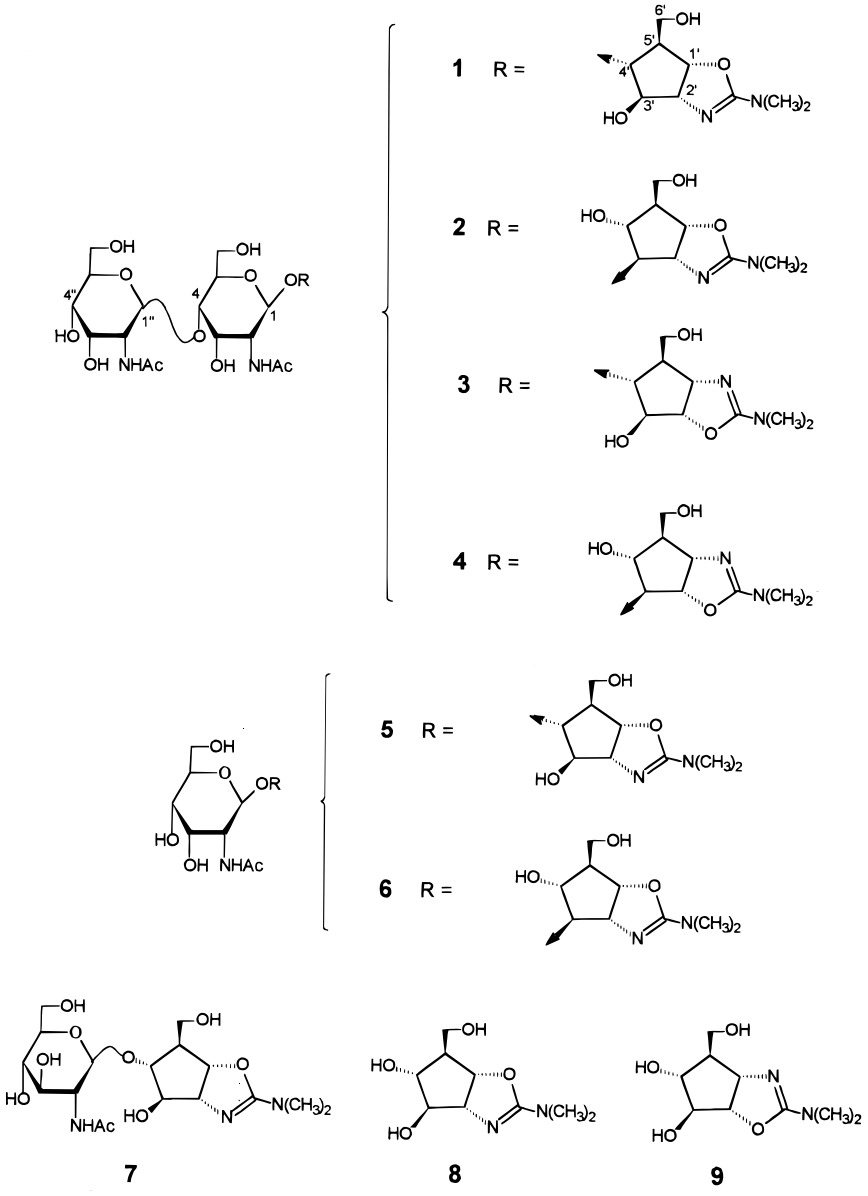

Fig. 1. Structures of compounds **1**–**9**.

undergoing moulting, a process which requires enzyme-catalysed depolymerisation of cuticular chitin.[22] Interruption of moulting by inhibition of the crucial chitinase might therefore form the basis of a new type of insecticide. We have synthesised allosamidin (**1**) and its analogues **2**–**9** (Fig. 1) and examined their biological activity, with the aim of gaining information on structure–activity relationships to be used in the discovery of a simple but potent new ecdysis inhibitor.

2 MATERIALS AND METHODS

Experimental details for the synthesis of compounds **1**–**9** have been published,[6,23] and a description of the chitinase inhibition experiments will be published shortly (Spindler-Barth, M., Blattner, R., Spindler K.-D. & Vorgias, C. E., unpublished results).

2.1 Insect cultures

The *Lucilia cuprina* (Wiedemann) culture was obtained from the Wallaceville Animal Research Centre. Pupae from mixed field strains, supplied in Petri dishes containing Vermiculite®, were held under controlled environmental conditions (25·0(±0·2)°C, 16 : 8 h light : dark, 50–60% relative humidity). Emerged adults were fed sugar and water *ad libitum*. Eggs laid on ox liver were incubated at 25·0(±0·2)°C and first-instar larvae were used for assays within two hours of hatch.

The *Tineola bisselliella* (Hummel) culture, originating from wild populations collected in 1994, had been cultured under controlled environmental conditions

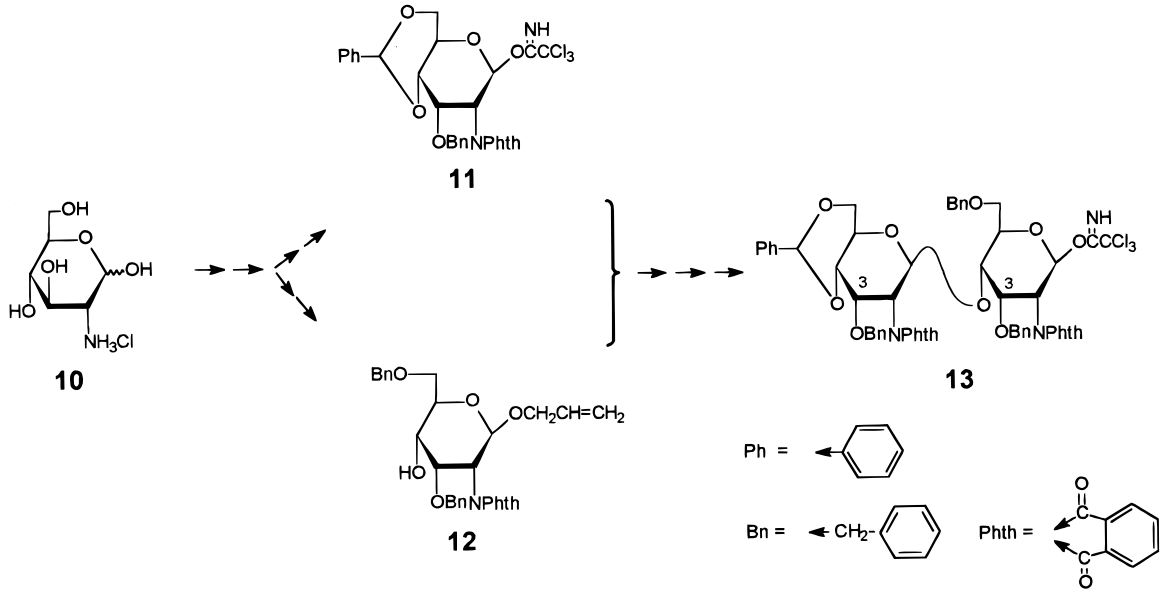

Fig. 2. Synthesis of disaccharide glycosyl donor **13**.

(25·0(±0·2)°C, 16 : 8 h light : dark, 50–60% relative humidity) in the absence of insecticides. Larvae were reared on a mixture of 90% casein, 9% yeast and 1% cholesterol and used at 19–21 days after hatch.

2.2 Insect bioassay techniques

2.2.1 Lucilia cuprina

The method was based on Levot.[24] Each test compound was dissolved in distilled water to give an approximately 2×10^{-2} M solution. Strips of Whatman grade 1 chromatography paper (30 × 60 mm) were treated with test solution (180 μl). Control strips were treated with distilled water (180 μl). Each treatment was replicated five times. Strips were rolled and placed in 50 × 12 mm glass vials. Forty (±2) newly hatched larvae were placed into each vial along with fortified (20 g litre^{-1} yeast extract and 5 g litre^{-1} KH_2PO_4) sheep serum (400 μl). Vials were plugged with non-absorbent cotton wool and held for 24 h in constant light at 25·0(±0·2)°C.

Contents of the vials were then washed with warm water into a glass dish. To asses mortality, the dish was placed on a dark background and the number of active larvae counted. Immobile larvae were assumed dead. Larval size and appearance were noted. Larval instar was assessed by placing the dish on a light background. The first and second instars could be readily differentiated, primarily by the size and definition of mouth parts, but also by spine visibility.

2.2.2 Tineola bisselliella

The method was based on the International Wool Secretariat assay for insect resistance.[25] Allosamidin (5 mg) was dissolved in distilled water (1 ml). Four squares of woollen cloth (2·5 × 2·5 cm, standard abradant fabric, mean weight 167 mg) were each treated with this solution (200 μl) to give a dose of 6 mg g^{-1} fabric. They were then left to dry overnight at ambient temperature. Controls were untreated cloth squares. Treated and untreated cloth squares were placed in individually labelled 150-ml plastic pots with well-

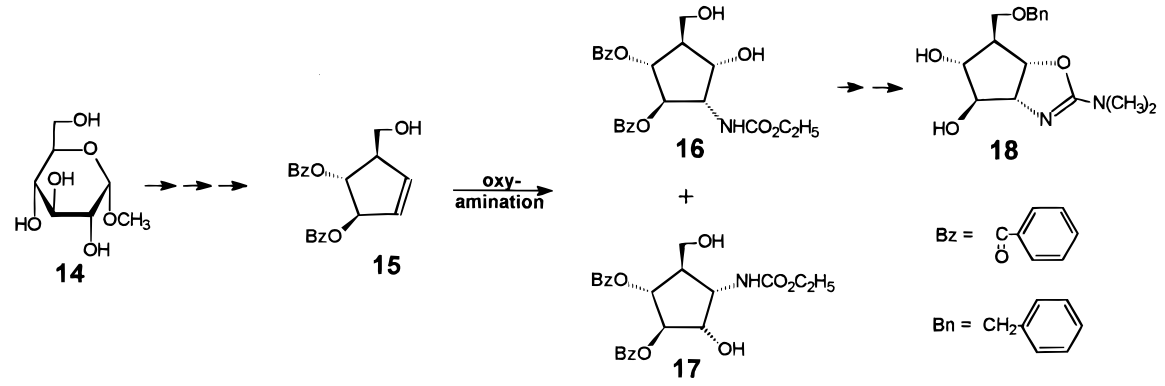

Fig. 3. Synthesis of cyclitol glycosyl acceptor **18**.

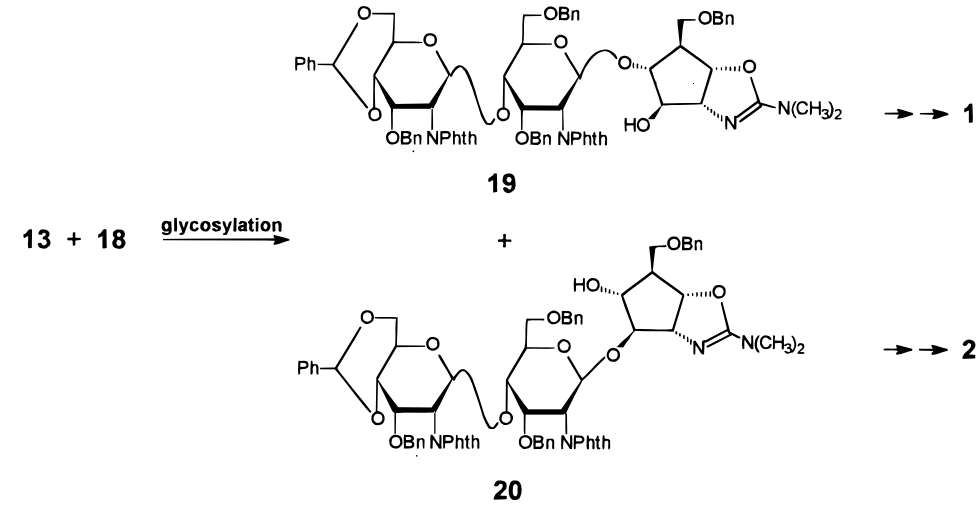

Fig. 4. Synthesis of allosamidin (**1**) and analogue **2** from disaccharide glycosyl donor **13** and cyclitol acceptor **18**.

ventilated lids. Fifteen *T. bisselliella* larvae were introduced into each pot and were held in a controlled environment room at 25·0(±0·2)°C, with 60% relative humidity. After 14 days, numbers of live, moribund, dead and abnormal larvae, and successful moults were assessed. Larvae were regarded as moribund when they failed to right themselves after being placed on their dorsal surface. Abnormal larvae were those with visible moulting abnormalities such as double head capsules (old head capsule retained over new head capsule). Successful moults were estimated by number of moulted head capsules. Larvae alive at bioassay assessment were replaced in the test containers and re-examined eight days later.

2.2.3 Statistics

Results were analysed by regression analysis. Differences between means were tested using Dunnett's LSD test.

3 RESULTS

3.1 Synthesis of test compounds

Our total synthesis of **1**,[6] outlined in Figs 2–4, comprised: (i) the preparation of disaccharide glycosyl donor **13** in 15 steps from glucosamine hydrochloride (**10**), *via* the monosaccharide glycosyl donor **11** and acceptor **12** (Fig. 2, 6·5% overall yield); (ii) the preparation of cyclitol glycosyl acceptor **18** in 11 steps from methyl α-D-glucopyranoside (**14**) by way of the functionalised cyclopentene **15**[26] and its oxyamination product **16** (Fig. 3, 8% overall yield); (iii) coupling between **13** and **18** to furnish trimer **19** which was deprotected to give **1** (Fig. 4, four steps, 27% overall yield).

Intermediates and by-products of this synthesis were then used to obtain a series of analogues. Since the cyclitol acceptor **18** is a diol, its reaction with the disaccharide donor **13** had furnished the (1 → 3)-linked

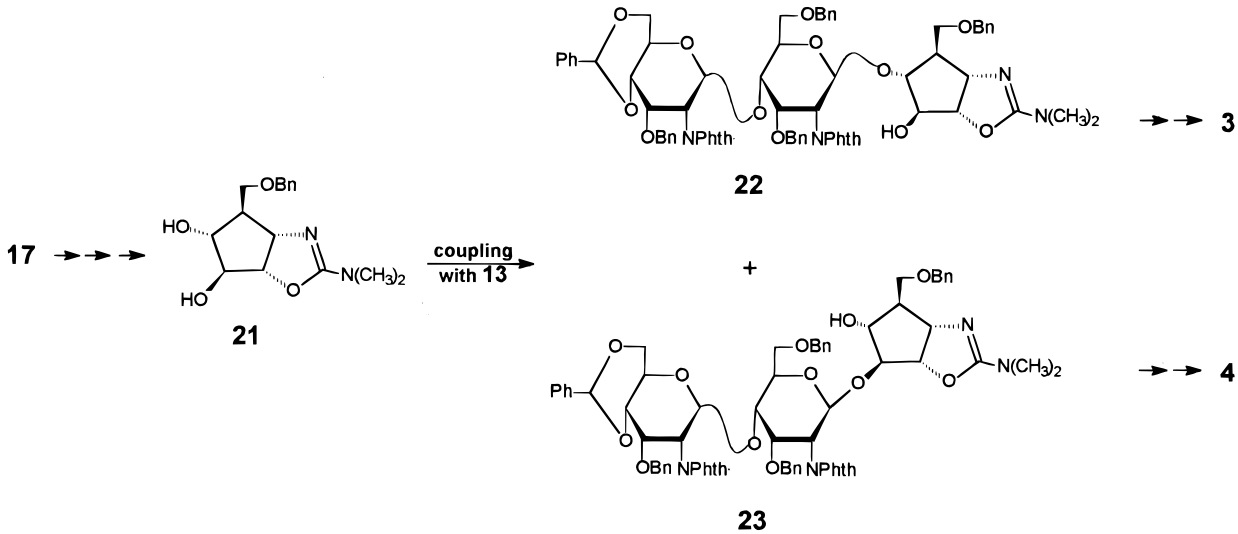

Fig. 5. Synthesis of analogues **3** and **4**.

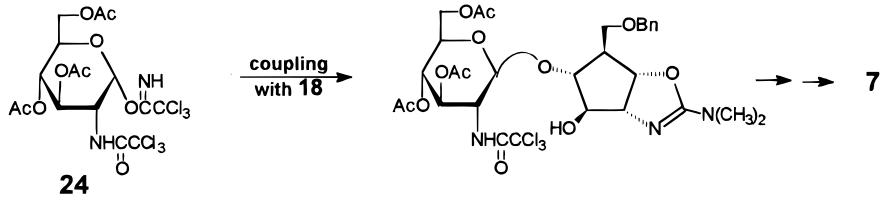

Fig. 6. Synthesis of analogue **7**.

trimer **20**, besides the required (1 → 4)-linked main product **19** (Fig. 4; ratio **19 : 20** = 4 : 1). The two isomers were easily separated by flash chromatography and deprotection of **20** gave the linkage isomer **2** of **1**.[6]

Considerable proportions of a minor product, the regioisomer **17**, had also been formed in the oxyamination of alkene **15** (Fig. 3).[6] This was converted to the diol **21**, a regioisomer of acceptor **18**, and hence to the trimers **22** and **23**,[23] following the procedures developed in the original work (Fig. 5).[6] Unfortunately, compounds **22** and **23** could not be separated by chromatography, but fractional crystallisation afforded a sizeable sample of the major, (1 → 4)-linked isomer **22** which was deprotected to the allosamidin regioisomer **3**, whereas deprotection of the mother liquors yielded a 1 : 1 mixture of **3** with the regio/linkage isomer **4**. The dimeric analogues **5** and **6** of **1** and **2**, respectively, were readily available by reaction of diol **18** with the monosaccharide glycosyl donor **12** and subsequent deprotection,[23] and the *gluco*-configurated dimeric analogue **7** was prepared by coupling of diol **18** with the known glycosyl donor **24**[27] followed by deprotection (Fig. 6; Blattner, R., unpublished results). Compounds **5** and **7** have been obtained previously by mild acid hydrolysis of allosamidin (**1**) and a natural analogue, respectively.[14] Finally, debenzylation of the two diols **18** and **21** gave the free cyclitols allosamizoline (**8**) and its regioisomer **9**, respectively,[23] which may be regarded as monomeric allosamidin analogues.

TABLE 1
Effect of Allosamidin (**1**) and Analogues **2**–**9** on Development and Mortality of *Lucilia cuprina* Larvae[a]

	Mean percentage of larvae (±*SE*)			
Treatment	*1st instar*	*2nd instar*	*Dead*	K_i (μM)[b]
Control	4 (±2)	95 (±2)	1 (±1)	—
1	4 (±3)	9 (±5)	88 (±6)	0·22
2	4 (±1)	94 (±2)	2 (±1)	16·9
3	5 (±2)	94 (±3)	1 (±1)	30·8
3 + **4** (1 : 1)	2 (±1)	2 (±1)	95 (±2)	—
5	2 (±1)	3 (±2)	95 (±3)	0·35
6	5 (±2)	94 (±3)	1 (±1)	62·9
7	2 (±1)	23 (±5)	75 (±5)	0·67
8	3 (±1)	96 (±2)	1 (±1)	234
9	1 (±1)	97 (±2)	2 (±1)	>5500

[a] Five replicates, 40 ± 2 larvae per vial.
[b] K_i values for inhibition of *Chironomus tentans* chitinase (Spindler-Barth, M., Blattner, R., Spindler K.-D. & Vorgias, C. E., unpublished results).

3.2 Bioassays

3.2.1 Lucilia cuprina

None of compounds **1**–**9** retarded the rate of development of *L. cuprina* larvae during the first instar, as shown by the lack of significant differences in first-instar larval numbers between samples and controls (Table 1). High larval mortality compared to controls resulted when *L. cuprina* larvae were exposed to allosamidin (**1**) ($P < 0{\cdot}01$), the dimeric analogue **5** ($P < 0{\cdot}01$), the regio/linkage isomer **4**, as a 1 : 1 mixture with **3**, ($P < 0{\cdot}01$) and the *gluco*-configurated dimeric analogue **7** ($P < 0{\cdot}01$). Dead larvae appeared as small, second instars. In some the retained first instars, exuviae were visible, encasing the larvae. The regioisomer **3**, and linkage isomer **2** and its dimeric analogue **6** showed no activity. Neither did allosamizoline (**8**) and its regioisomer **9**. These results are consistent with K_i values for the inhibition of insect chitinase from *Chironomus tentans* (Spindler-Barth, M., Blattner, R., Spindler, K.-D. & Vorgias, C. E., unpublished results) which are included in Table 1.

TABLE 2
Effect of Allosamidin (**1**) on Mortality, Abnormal Moulting and Mean Number of Moults by *Tineola bisselliella* Larvae[a]

	Mean mortality (%) (±*SE*)			
Treatment	*14 days*	*22 days*	*Abnormalities* (%) (±*SE*)	*No. moults*
Control	2 (±2)	2 (±2)	0	12
1	63 (±7)	80 (±7)	49 (±5)	10

[a] 15 larvae per replicate.

3.2.2 Tineola bisselliella

Allosamidin (**1**) showed activity against *T. bisselliella* larvae (Table 2). Significantly fewer live larvae ($P < 0{\cdot}01$) and higher numbers of abnormal larvae ($P < 0{\cdot}01$) were found on treated than on untreated fabric. Retardation and interruption of moulting was evident. High numbers of larvae in the process of moulting were present on the treated fabric (head capsule clear with rear dorsal edge free of prothoracic shield). These included live, moribund and dead larvae with an abnormal, turgid, stiff appearance. Observations of larvae retained for eight days after assessment indicated the moult was completed eventually only by those with a normal appearance. The amount of (**1**) ingested could not be calculated as larval wool consumption was not assessed.

4 DISCUSSION

Apart from two brief reports at the time of the discovery of the parent compound (**1**)[1,2] this is the first account, as far as we know, of the effects of allosamidins on insects after oral and/or contact administration. These in-vivo observations are consistent with the findings that compounds **1**, **5** and **7** are the most active inhibitors of insect chitinase (Spindler-Barth, M., Blattner, R., Spindler, K.-D. & Vorgias, C. E., unpublished results). They also fit the model derived from an X-ray structural analysis of allosamidin complexed to the binding cleft of hevamine,[28] a plant chitinase with catalytic domain very similar to that of insect chitinases.[29] Thus, the biological activity was not affected by the absence of the second *N*-acetylallosamine unit (compound **5**) and only slightly reduced by the altered orientation of the hydroxyl group at C-3 of the remaining sugar residue (compound **7**) which does not appear to be involved in hydrogen-bonding to the enzyme.[28] Structural modifications which changed the spatial arrangement of the cyclitol part, on the other hand, i.e. linkage to the saccharide unit at the 'wrong' position (compounds **2** and **6**) or change of place between nitrogen and oxygen (compound **3**) destroyed the activity. In the complexed allosamidin (**1**), the nitrogen atom of the oxazoline ring, protonated and positively charged under optimal pH conditions, is bound at a position which showed preference for a positive charge.[28] In the linkage isomers **2** and **6** and in the regioisomer **3**, the ring nitrogen atom has been removed from this position. When both these modifications are applied at the same time, however, the nitrogen atom may be able to occupy the same location in the active site as in the original molecule **1**; this would explain the high activity, rather surprising at first glance, of the linkage/regioisomer **4**, tested as a 1 : 1 mixture with **3**, which is itself not active.

5 ACKNOWLEDGEMENTS

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